

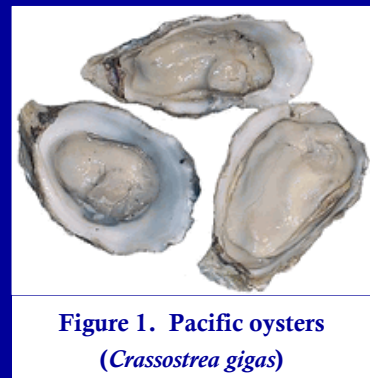
Effect of age and tissue weight on the cadmium concentration in Pacific oysters (*Crassostrea gigas*)

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Introduction

Cadmium (Cd) is a trace metal found in soil, air, water and living things. It can be released naturally from mineral deposits or as a result of anthropogenic activity, including metal smelting, use of phosphate fertilizers and burning of fossil fuels. Cd can also be found in some batteries, pigments, metal coatings and plastics. After its release into the environment, Cd can accumulate in plants (e.g. tobacco leaves, green leafy vegetables) or in the soft tissues of both humans and animals. It has a long biological half-life (10-30 yrs), and long-term exposure can result in health problems such as kidney dysfunction, liver disease, lung cancer and skeletal decalcification (Nordberg et al. 2003; Satarug et al. 2006). Humans are primarily exposed to Cd through smoking tobacco and food consumption (Kikuchi 2002; Satarug and Moore 2004).



Bivalves, including Pacific oysters (*Crassostrea gigas*) (Fig. 1), can accumulate metals such as cadmium in their soft tissues due to several inherent biological properties. For one, they filter large volumes of sea water and can therefore be exposed to high levels of dissolved and particulate-sorbed pollutants. They also contain the protein metallothionein, which binds and sequesters metals (Geret et al. 2002; Perceval et al. 2002). Although metallothionein reduces the toxic effects of metal exposure, it also allows for survival of organisms carrying elevated body burdens of toxicants, including cadmium.

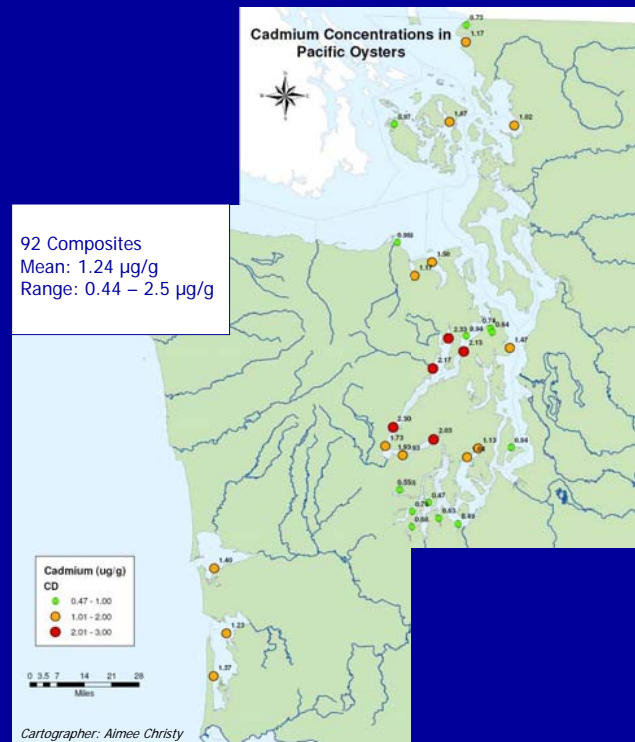


Figure 2. Cadmium (Cd) concentrations in Pacific oysters sampled at various growing beds throughout Washington (Christy, 2005).

Recently, shipments of Pacific oysters from Washington were rejected by Hong Kong because they contained levels of Cd that exceeded the import standard of 2.0 µg/g (ppm) wet wt. These rejected shipments pose an economic threat to the Pacific Northwest oyster industry and raise human health concerns. Therefore, the Oregon State University Seafood Laboratory, in collaboration with Pacific Shellfish Institute, California Sea Grant (UC Davis), Integral Consulting, Hong Kong University of Science and Technology and University of Alaska, initiated a USDA-funded project with the following goals:

1. Evaluate the spatial distribution of Cd in West Coast Pacific oysters (Fig. 2).
2. Identify factors that may influence Cd concentrations.
3. Evaluate the impact of Cd on the shellfish industry and human health.
4. Provide outreach and extension services.

As part of the response to the 1st goal, a comprehensive sampling effort was carried out in which composite oyster samples from various growing sites in Washington, Alaska and Oregon were analyzed for Cd (Fig. 2) (Christy, 2005). The most extensive sampling was carried out in Washington, in which 92 composites had an ave. Cd concentration of 1.24 ppm, with 17% of the composites in excess of 2 ppm. In the work presented here, further research into the effects of biological factors on Cd levels in oysters was carried out at one of the WA growing sites. This work was performed in response to the 2nd research goal listed above.

Objectives of present study:

Examine the effects of age and tissue weight on US West Coast Pacific oysters.

Materials and Methods

Age Study

- Oysters from 4 age groups (1, 2, 3, and 4 yrs) and corresponding were collected from an oyster farm August 2005 in Washington.
- Pre-determined shell size ranges for each age group were as follows:
 - 1 yr olds: 2.5-4.5 in.
 - 2 yr olds: 3.5-5.5 in.
 - 3 yr olds: 4-6 in.
 - 4 yr olds: 5.5-8 in.
- A pre-established sampling protocol (Fig. 3) was used with 3 composites of 20 oysters per age group.
- Oysters were washed, shucked, homogenized in composites of 20, and weighed prior to analysis.
- Homogenized oyster composites and sediment samples were analyzed for Cd concentrations using inductively coupled plasma-atomic emission spectroscopy (ICP-AES), EPA Method 6010.

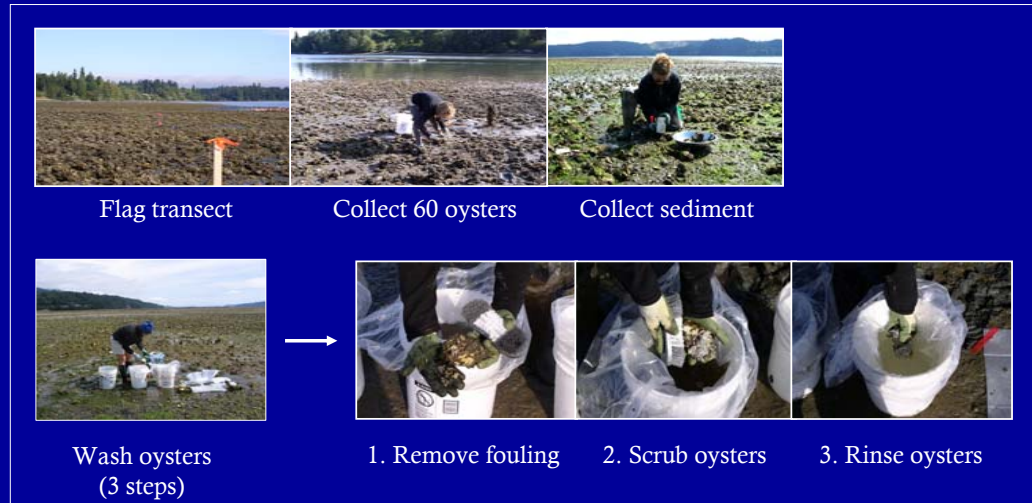


Fig 3. Sampling collection methods for Pacific oysters used in age study.

Tissue Weights Study

- 100 oysters from 4 age groups (1,2,3 and 4 yr olds) were collected in August 2005 from an oyster farm in Willapa Bay, Washington.
- 25 oysters from each age group were washed, shucked, weighed, and individually analyzed for Cd concentration by ICP-AES, EPA Method 6010.

Results: Age Study

As shown in Table 1 and Fig. 4, all oyster cadmium concentrations were below the 3.7 ppm level of concern set by the Food and Drug Administration (FDA) and the 2 ppm Hong Kong import standard. The 1-yr old oysters had a significantly ($p < 0.05$) lower Cd concentration (0.75 ppm) compared to the older age classes. Although there was no statistical difference among the 2-, 3- and 4-yr olds, there were still slight increases in Cd concentration with each successive increase in yr class, with ave. Cd concentrations of 1.04, 1.06 and 1.11 ppm, respectively. These results indicate that age does have some influence on Cd concentration, especially during the first few years. In terms of weight, there was no statistical difference between the 1- and 2-yr olds; however, both the 3- and 4-yr olds were significantly heavier ($p < 0.05$). Sediment Cd concentrations ranged from 0.26-1.10 ppm, with no strong correlation to ave. oyster Cd levels.

Table 1. Results of oyster age study showing yr class, ave. oyster Cd, tissue wt. and sediment Cd.

| Age (yr) | Ave. Cd (ppm) and range | Ave. oyster wt. (g) | Sediment Cd (ppm) |
|----------|---------------------------------------|-------------------------|-------------------|
| 1 | 0.75 ^a ± 0.03 0.72-0.77 | 16.7 ^a ± 1.2 | 0.39 |
| 2 | 1.04 ^b ± 0.13 0.90-1.16 | 19.9 ^a ± 0.9 | 0.26 |
| 3 | 1.06 ^b ± 0.04 1.03-1.10 | 41.7 ^b ± 2.3 | 0.94 |
| 4 | 1.11 ^b ± 0.11 1.04-1.24 | 79.5 ^c ± 1.7 | 1.10 |

Note: Values in the same column labeled with a different superscript letter are significantly different ($p < 0.05$) according to one-way analysis of variance (ANOVA), Tukey's test.

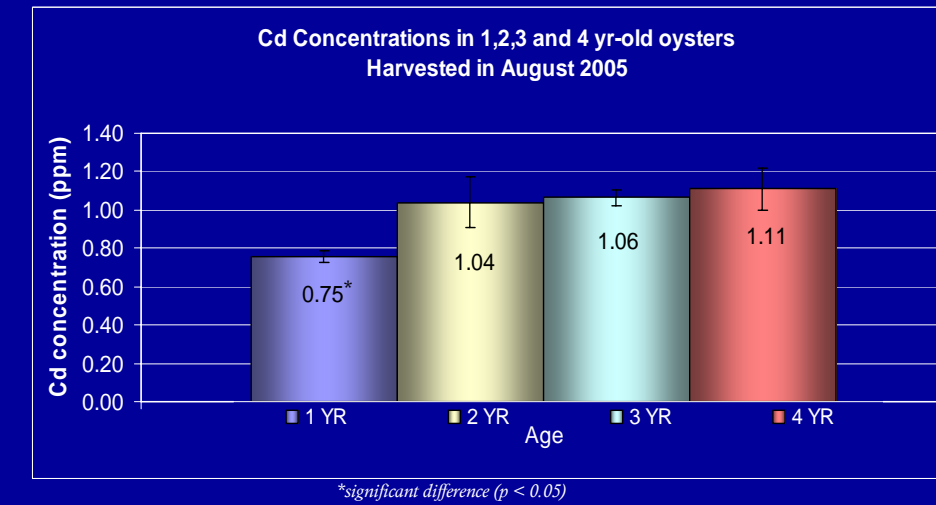


Figure 4. Comparison of the Cd concentrations in oysters grouped by age class.

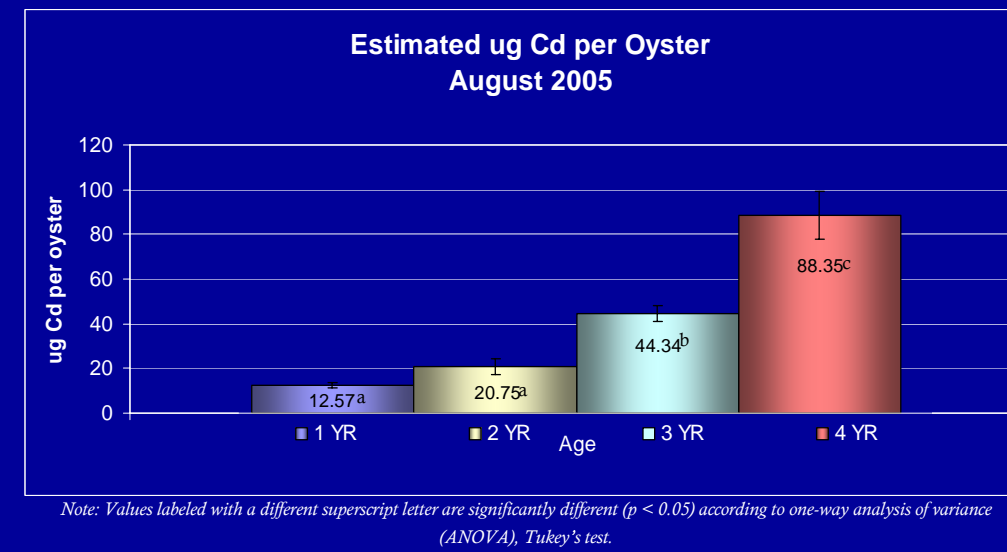


Figure 5. Estimated micrograms (ug) of Cd per oyster according to age class.

There was no significant increase in estimated ug Cd/oyster among the 1- and 2-yr old oysters (Fig. 5); however, there were significant increases for the 3- and 4-yr olds ($p < 0.05$). The US Environmental Protection Agency (EPA) has established a reference dose (RfD) that allows for a 70 kg individual to eat up to 70 ug Cd/day (490 ug/wk) (EPA, 1994). Based on the results presented here, this is equivalent to about 40 1-yr olds, 23 2-yr olds, 11 3-yr olds, or 5 4-yr olds per wk.

Results: Tissue Weights Study

As shown in Fig. 6, there was some effect of oyster weight on Cd concentration, with an R-squared value of 0.37 for all 100 oysters analyzed in the tissue weights study. Interestingly, when the oysters were separated by yr class, it was apparent that the relationship between weight and Cd concentration varies with age (Fig. 7). There was a moderate correlation for the 1- and 2-yr olds ($R^2 = 0.29$ and 0.41 , respectively), while the R-squared values for the 3- and 4-yr olds dropped to 0.11 and 0.03, respectively. An interpretation of Fig. 7 suggests that the oysters accumulate Cd as they grow during the first two years of life, with the cadmium concentration possibly reaching a saturation point after 3-4 yrs of growth. These differences might also be related to physiological changes and/or variations in food preference.

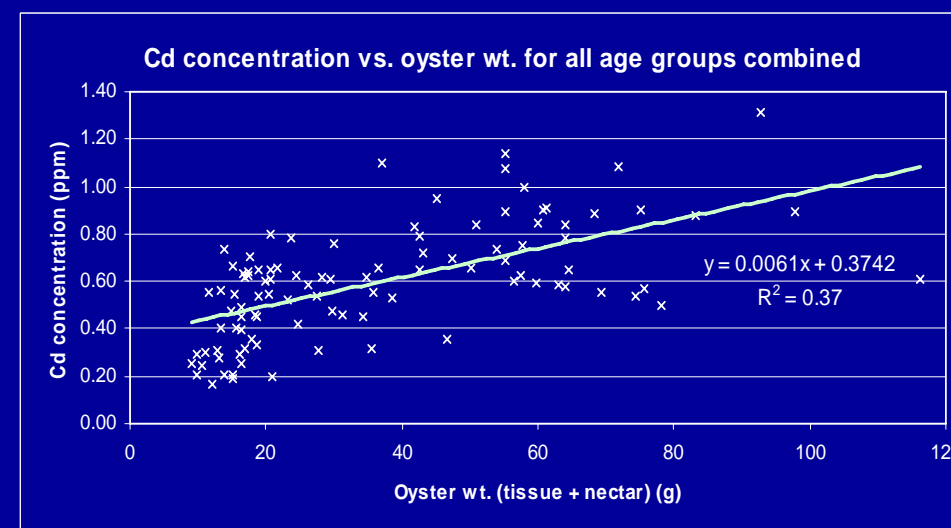


Figure 6. Relationship between Cd concentration and individual oyster weights (tissue + nectar).

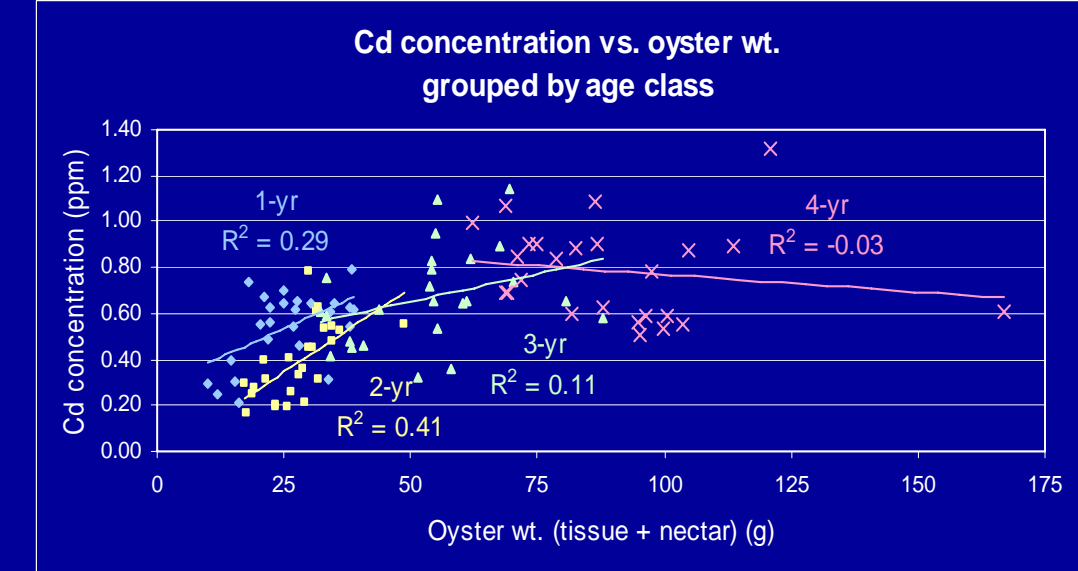


Figure 7. Relationship between Cd concentration and individual oyster weights (tissue + nectar) grouped by age class.

Conclusions

- **Age Study**
 - There was some effect of age on Cd concentrations: 1-yr old oysters had significantly lower ($p < 0.05$) Cd concentrations as compared to 2-, 3- and 4-yr olds.
 - 1- and 2-yr olds were fairly close in weight and in estimated total Cd (ug); however, these parameters doubled at ages 3 and 4 yrs.
 - Sediment Cd levels did not appear to have a direct influence on oyster Cd concentrations.
- **Tissue Weights Study**
 - There was an effect of oyster tissue weight on Cd concentrations, with an R-squared value of 0.37 for all samples combined (N = 100).
 - The relationship between tissue weight and Cd concentration was found to vary with age, with moderate correlations for the 1- and 2-yr olds, a weak correlation for the 3-yr olds, and practically no correlation for the 4-yr olds.
- **Overall Conclusions**
 - All oyster Cd concentrations were below the 3.7 ppm FDA level of concern and the 2 ppm Hong Kong import standard.
 - Results indicate that oysters accumulate Cd as they grow during the first two years of their lives, after which point the Cd concentration may reach a saturation point during the 3rd and 4th years of life. Whether this is due to physiological activity or diet remains to be investigated.

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Acknowledgements

This work was funded by the US Department of Agriculture (USDA) Nationally Integrated Food Safety Initiative.